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Review

Past, present, and future of HPV research: highlights from the 19th International Papillomavirus Conference-HPV2001

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Abstract

This manuscript summarizes the papers presented at the 19th International Papillomavirus Conference, held in Florianopolis, Brazil, September 1–7, 2001, divided in four main areas: Clinical diagnosis and screening, Epidemiology, Biology and Immunology. It provides an overview of what we know about the biology and life cycle of these viruses, their interaction with human and animal hosts, and the diseases that they cause. Highlights derive from the analysis of more than 500 papers presented at the Conference.

Keywords: Human papillomavirus (HPV); Epidemiology; Biology; Immunology; Cancer prevention; Clinical diagnosis

1. Introduction

The 19th International Papillomavirus Conference was held in Florianopolis, south of Brazil, from September 1st to 7th, 2001. The meeting attracted about 700 people, among physicians and researchers of 39 countries around the World. Overall, 514 papers were presented 202 as oral presentations (O-references) and 312 as posters (P-references). The full text of the abstracts and lectures below refer to the abstract number on the conference's website http://www.hpv2001.com; abstract books can be obtained upon request to llvilla@ludwig.org.br. The highlights of the conference were selected and are briefly reported below.

The keynote address was presented by Professor Harald zur Hausen of the German Cancer Research Center in Heidelberg, Germany. He gave an extensive overview about the present understanding of mechanisms of viral carcinogenesis. Emphasis was given to the involvement of cellular signaling cascades in the control of viral gene

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regulation that were compared with the significant amount of related information accumulated in papillomavirus research. It became apparent the large contribution that papillomavirus research, including cutaneous Human papillomaviruses (HPVs), is making to our understanding of virus-linked cancers.

As in previous meetings, a clinical workshop preceded the main conference aiming at the transfer of knowledge from bench to bedside. The first part of the program concentrated on the epidemiological and molecular background that supports the etiological role of HPV in cervical cancer (O-2, O-3 presented by H-U. Bernard, O-4–O-6). The importance of evidence-based medicine was highlighted by two talks (O-1 and O-7) who provided a critical view of how HPV testing, screening colposcopy and other measures could be considered in defining evidence-based care with regard to cervical cancer.

Diagnosis and management of precursor lesions, namely low- and high-grade squamous intraepithelial lesions (SIL) was approached by several speakers (O-8–O-11; O-21–O-24). As a general conclusion, evidence was presented for the safety of a conservative approach when considering L-SIL, due to its low potential for progression and elevated rates of regression. Moreover, the value of HPV typing as a predictor of disease progression was discussed. A critical evaluation of morphological (O-12) and molecular (O-13), including serological (O-14) methods to diagnose HPV infections was presented.

A full session was dedicated to screening strategies against cervical cancer. Evidence for the efficacy of cancer screening methods was presented in relation to cytologic screening (O-26) as well as for direct visual inspection of the cervix with acetic acid (VIA), particularly in low-resource settings (O-27). A general evaluation of benefit for cancer-screening methods was discussed based on randomized controlled trials with proper end points (O-25).

Two sessions were devoted to new approaches in the prevention of cervical cancer. The perspective of the World Health Organization and its cancer control agency, the International Agency for Cancer Research (IARC), concerning the development of an HPV vaccine were presented (O-29 and O-30). Several studies and recommendations were proposed in order to prepare and launch vaccination programs in under-served populations. The experience from low-resource settings concerning screening services and strategies for HPV testing in self-collected samples was presented (O-31 and O-32). The problem of cost-effective screening strategies for the under-served populations was presented in several talks. The use of methods based in self-collected samples and the study of strategies and methods to evaluate and treat women in one step (see-and-treat) are considered useful alternatives. The importance of application of quality assurance in each step of the screening strategies was presented as a mean to improve the effectiveness of cervical cancer screening programmes (O-33).

A series of oral presentations were devoted to Vaccine Development that has clearly shown the important progress in this field (O-50–O-65). The session on Prognostic factors and New therapeutic approaches to cervical cancer was introduced by a very comprehensive talk (O-72) followed by several abstracts.

In the main conference two sessions were devoted to Clinical Diagnosis and Screening but a number of presentations dealing with diagnosis and therapy issues were included in sessions about Epidemiology, Immunology and Biology. The differential expression of $P16^{INK4A}$ in cervical and vulvar intraepithelial lesions was considered as a potential tumor marker and new diagnostic tool, adding new options to the screening strategies (O-67, O-70 and O-71).

Several talks and posters were presented in the Clinical Diagnosis and Screening sessions. The value of HPV detection and typing was evaluated in several clinical contexts. It role as a predictor of HGSIL recurrences after excisional therapies could reduce the number of visits, the number of tests and the length of follow up.

As in previous reports, single detection of high-risk types of HPV was more sensitive and less specific than cytologic screening for the identification of subsequent CIN 3 diagnosis. However, the combination of both techniques did not significantly improve the performance of HPV test. By

the other side, persistent positivity of high-risk types of HPV has clearly stood as a major risk factor for the development of HGSIL, with very high relative risk levels. In the same way, viral load, as a surrogate for HPV persistence, seems to be useful in identifying women at a higher risk of developing cervical cancer.

In the field of therapy, immunotherapy and specially prophylactic and therapeutic vaccines, have been extensively studied as very promising tools for preventing and treating not only HPV infection but also cervical dysplasia. This topic has been analyzed in details in the Immunology session, as described below.

Many questions are still to be answered such as: (1) Would the use of HPV testing increase the frequency of HGSILs in screening programmes? (2) Will enhanced detection lead to a decrease in incidence of and mortality from cervical cancer? (3) Can HPV testing be used to safety lengthen screening intervals? (4) Will HPV testing be more cost-effective than cytologic primary screening? (5) Can immunotherapy of intraepithelial lesions decrease the incidence of cervical cancer? (6) Should we treat LGSILs containing high-risk HPV types? (7) Should we use strategies of screen and treat patients at the same visit in low resources areas? To answer these questions we need more longitudinal studies and, especially for questions about interventions, we need more Randomized Controlled Trials, which can provide the best quality of evidence.

At the **Epidemiology** sessions held during the 19th International Papillomavirus Conference, results were presented from large, prospective studies designed to confirm the etiological link between infection with oncogenic types of HPV and the development of cervical neoplasia. As expected, these studies clearly confirmed the HPV-cervical cancer link (O-89, O-212). Some data are now emerging to suggest that HPV16 behave differently from all other oncogenic and non-oncogenic HPV types (O-133, P-219, P-237). Data from one longitudinal study suggested that over a period of 5 years, the absolute risk of progression of HPV16 infection to high-grade disease and cancer is very high (approximately 50%). Among HPV16 infections that do not progress, the vast majority regresses. Thus HPV16 persistence in the absence of progression was rarely seen. Similarly, studies among HIV+ and HIV− groups indicate that the relative increase in HPV16 prevalence in HIV+ compared with HIV− women is much smaller than that for other HPV types. This suggests that immune suppression does not affect HPV16 as much as other types, and indirectly indicates that HPV16 has the ability to evade the immune system even in immune competent individuals.

The relationship between viral variants, viral load and disease risk was also discussed extensively at the meeting (O-66, O-96, O-155–O-160, O-194, P-155, P-182). New data continue to support the notion that non-European variants of HPV16 confer higher risk of cervical cancer than does the European variant of HPV16. Similar data are beginning to emerge for other HPV types, including HPV18. Cautionary notes in this area include the need to rule out the possibility of confounding by ethnicity as a partial explanation for some of the findings and the interest in examining possible recent shifts in variant distribution that might account for case-control findings.

Several studies evaluated the possibility that HPV viral load is a predictor of disease development (O-81, O-93, O-134, P-209, P-235, P-241). Results presented from cross-sectional studies appear conflicting, with some suggesting increasing viral load with increasing disease severity and others pointing to evidence that the highest viral load levels are observed for LSILs. Preliminary findings from longitudinal studies suggest an association between viral load and disease progression, but the exact patterns of such risk remain elusive. Discrepancies between studies might partially be explained by the lack of standardization in the assay methodologies used to measure viral load, and also by difficulties in avoiding sample collection variability. Future efforts to standardize viral load assays will be important in this regard.

In addition to HPV itself, numerous presentations focused on co-factors that may be important in the etiology of HPV-related tumors (O-79, O-90, O-131, O-135, O-188, O-189, O-193, P-193, P-206, P-223, P-224, P-227, P-236). Two co-factors now appear to be clearly associated with cervical

cancer: parity and cigarette smoking. The parity association, in particular, is strong, but the biological mechanism(s) for such an effect are poorly understood. Evidence (largely from cross-sectional studies) suggests a possible link between long duration of oral contraceptive use and cervical cancer risk. Whether these findings will be confirmed in longitudinal studies remains to be determined. Of the 'standard' cervical cancer cofactors evaluated over the years, sexually transmitted diseases other than HPV remain the most controversial. Data on both HSV-2 and *Chlamydia trachomatis* studies were presented. Differences between case-control and cohort study results have resurfaced old discussions of which design is best suited to evaluate the association with cervical cancer of STDs that change over time. Concerns over residual confounding by HPV when examining other STDs were also expressed.

Data on male factors was also presented at the meeting (O-85, O-88, O-136). In particular, interesting data on the effect of circumcision of the male partner on risk of cervical cancer were reported. Based on the data presented, there, now appears to be clear evidence for a protective effect of male circumcision on risk of cervical cancer in their partners.

Several groups presented data on HLA-disease associations (O-66, O-68, O-94, O-100, O-130, O-132, P-138, P-190). A consistent pattern has appeared suggesting reduced risk of disease associated with HLA DRB1*1301. Less clear patterns are seen for HLA alleles hypothesized to increase disease risk, possibly because carriage of multiple risk alleles may be required to confer measurable effects. Efforts are now underway to combine individual HLA alleles into biologically relevant categories that might increase the power of epidemiological studies to evaluate risk associated with HLA. In addition to the well studied HLA Class II alleles, data presented at the meeting suggest that class I alleles might be of interest to study. The lack of HPV type-specificity, in the HLA Class I associations reported suggest a role of innate immunity and possibly of natural killer cells that recognize HLA Class I alleles.

Host immunological responses to HPV are believed to play an important role in disease development. However, the exact mechanisms involved in protection and markers of such effects are not well understood. At the meeting, methodological studies were reported that would increase the ability of epidemiological studies to evaluate markers of immune response. The first careful attempts to evaluate local immune response at the cervix within the context of epidemiological work were reported. Also, data presented by laboratory colleagues provided interesting clues for future epidemiological work. Among others, studies demonstrating that immune response to the HPV16 E2 protein may be more robust than those against other early proteins should be exploited. Demonstration of the polyclonality of antibody responses to HPV L1 might also have interesting epidemiological implications.

With respect to antibody responses, longitudinal studies have permitted an evaluation of whether natural levels of anti-HPV antibodies generated in response to infection protect against subsequent re-infection by the same or related HPV types (O-95, O-103, P-208). Data presented at the meeting suggest that HPV16 IgG antibodies do not protect against subsequent re-infection with the same viral type, but in at least one study reported at the meeting preliminary evidence was presented suggesting that IgG antibodies against HPV18 and HPV31 might protect against same-type re-infection. These results should not be interpreted as a suggestion that prophylactic vaccination against HPV will not be efficacious, since levels of antibodies generated in response to vaccination have been shown to be 40-fold higher, on average, than those observed after natural infection.

It is intriguing how much new information is still being gained about papillomaviruses, and how many aspects of their life cycle are not yet understood. Most research on the **biology of papillomaviruses** has been done with eight virus types, Human papillomavirus-6 (HPV-6), HPV-8, HPV-11, HPV-16, HPV-18, HPV-31, bovine papillomavirus-1 (BPV-1) and the Cottontail rabbit papillomavirus (CRPV), which together have about 60 000 nucleotides. Over the last 20 years, roughly 1000 scientists (including all students and technicians) have worked at any particular time on these viruses. Altogether, this represents 20 000 person

per years of research, which means that the scientific community spent 1 person per year for every 3 nucleotides. Yet, everyone feels that we still have a long way to go to fully understand many important details of the biology of these viruses. The below summary is based on 120 contributions.

2. Transcription and replication

Papillomavirus transcription and replication is modulated by the viral E2 and E1 proteins, and more than 100 host cellular proteins, as explained in an overview on this subject (O-35). Unfortunately, only a few labs are presently working on these cellular proteins. On this conference, Bromberg and colleagues (P-150) reported that HPV-31, just like HPV-16 and HPV-18, is induced by progesterone. The resulting up-regulation of E6 and E7 oncoprotein expression is a beautiful example of how epidemiological findings, namely the increase in the incidence of cervical cancer under the influence of multiple births and long-term oral contraceptive use, can be grounded on a molecular mechanism. Smola-Hess and colleagues (O-42) showed data that HPV-18 transcription could be stimulated by IL-6 in a STAT3 dependent manner, although the *cis*-responsive target has not yet been identified. One of the biggest enigmas of the PV field has been the difficulty to identify and functionally study the late promoters. This field is still in its infancy, however, there is increasing evidence that a promoter within the *E7* gene of HPV-31, and homologues in HPV-6 and HPV-16, are the late promoters of genital HPVs. Bodily and colleagues (P-141) have presented preliminary data on how to dissect this promoter.

Virtually every observation on the gene expression of non-genital papillomaviruses, e.g. BPV-1 and epidermodysplasia verruciformis associated (EV) HPVs, points to very different transcriptional strategies of these viruses. An example is the location of the late promoters of BPV-1 and EV-HPVs in the 5′-part of the long control region, and a strange sequence element, namely an extremely long AT stretch, at the E6 promoter of many EV-HPVs. In this conference we learned about further discrepancies between EV-HPVs and genital HPVs, as Akguel and colleagues (O-202) and Boeckle and colleagues (P-140) reported that transcription of HPV-8 is regulated by p53, the interferon regulating factor-5, RUNX1, and a potentially novel factor, PRF-1.

It is regularly observed that HPV genomes are integrated into the cellular chromosomes in cancer, but are episomal in precursor lesions. Many publications dealt with the consequences of this recombination, namely an interruption of repression by E2, and, according to our work, the activation of an enhancer depends on chromosomal recombination (O-35). Johannsson and colleagues (P-280) have reported on an HPV-33 positive cell line derived from a benign vaginal lesion, which progressed to a transformed phenotype during culturing. This transformation event coincided with the viral integration and resulted in the up-regulation of telomerase activity, adding further support to a model that integration is not only a byproduct but also a causal progression event. On the other hand, we learnt of the possibility that expression of integrated HPV genes can be turned off by CpG methylation, as observed for most of the HPV-16 copies of CaSki cells (Van Tine, O-128).

There are two types of repression mechanisms by E2: The full-length E2 protein can displace the cellular Sp1 and TFIID factors at the E6 promoter. However, E2 is expressed in genital HPVs also in form of an E8-E2 spliced protein, which can exert repression over long distances in an as yet little understood manner. Here, Zobel and colleagues (P-144) isolated two mutants, W6 and K7, of E8-E2, which relieve long-distance repression. Surprisingly, they also led to a 30–50-fold higher replication, possibly due to relief of repression of a viral promoter that is relevant for replication and normally repressed by E8-E2.

Another function of E2 is to tether the viral genomes to cellular chromosomes, which is important for segregation of the viral genome and copy number control. McBride and colleagues (O-39) reported that mutants defective in transactivation functions are also defective in association with mitotic chromosomes, an observation that may be useful to identify the protein targets that permit E2

to associate with chromosomes. This model was expanded by Voitenleitner and colleagues (O-166) with data on a phosphorylation defective mutant of E2 (A4), which is defective in binding chromosomes. Mutations in E2 or in E1 were identified that suppress the requirement for phosphorylation, suggesting that the E1 protein is playing some role in the interaction between E2 and chromosomes. At the present advanced stage of papillomavirus research, every virus-encoded protein is studied in the form of numerous point mutants. A beautiful example of the power of resolving the function of individual amino acid residues formed a study by West and colleagues (O-37) of the E1 protein of BPV-1.

It has been known for a number of years already, that in mucosal HPVs the recognition of two specific E2 binding sites by a complex of E2 and E1 proteins is more important for determining the replication origin than the recognition of the E1 binding site by E1 protein. At this conference, Lin and colleagues (O-38) confirmed the necessity of E2 for this recognition reaction; however, they found that E2 inhibits the subsequent unwinding reaction that is catalyzed by E1. Sheikh and colleagues (O-198) reported that in the cutaneous type HPV-1, in contrast to the mucosal HPVs, E2 plays only a supportive role in origin recognition, as E1 binding sites are sufficient for initiation of replication.

Over the years, many clinical studies reported that multiple HPV types could infect the genital tract at the same time, while it remains unclear, however, whether multiple HPV types can reside in the same cell in situ. In this conference, McLaughlin-Drubin and colleagues (P-148) reported that after transfection in vitro, two HPV types can stably replicate in the same cell, so there is no principle incompatibility in these viruses as in certain bacterial plasmids.

There have been previous reports that genital HPVs can replicate in mammalian cells in an E1 independent and yet poorly understood way. On this conference, it was one of the most surprising reports by Angeletti and colleagues (O-41) and Zhao and colleagues (P-273) that BPV-1 and several genital HPV types can stably replicate in yeast in the absence of any viral gene product. In future research, this may help to investigate cellular proteins required for episomal replication, and it may also become a useful technique to generate VLPs for vaccination purposes, as VLPs generated in yeast were reported here to be infectious in mammalian cell cultures.

3. The oncoproteins E5, E6 and E7

The multiplicity of the functions of the three small papillomavirus oncoproteins, E5, E6 and E7 continues to be amazing. Specifically, more than a dozen protein-protein-interactions between E6 and cellular proteins have been published. In this conference it was learned from Du and colleagues (P-288) of yet another E6 interaction partner, fibulin-1, which has been previously implicated in a variety of transformation and tumor invasion events. Fibulin-1 is located in the extracellular matrix, and its function is apparently impaired by E6, as over-expression of fibulin-1 reduced E6-mediated transformation. Research from Denise Galloway's lab (O-123) revealed that increased telomerase activity originates from transcriptional stimulation of the telomerase promoter by E6, probably through interaction with a presumed transcriptional repressor, NFX1. A transgenic mouse model of Paul Lambert's lab (O-122) led to evidence that the E6 protein continues to support carcinogenesis when present in a mutant form that poorly degrades p53, pointing toward the pathogenetic relevance of some of the other molecular functions of E6.

There are not yet as many molecular functions known of the E7 protein as for E6, and talks by Roesl and colleagues (O-169) and Fisher and colleagues (O-175) described the interactions of E7 with cdk2/cyclin A and E complexes and the consequences for cdk2 activity.

The E5 protein is traditionally known to interact with the *trans*-membrane domain of the EGF receptor and to modulate its concentration and phosphorylation. Fehrmann and Laimins (O-172) and Genther et al. (P-298) reported on E5 mutants in the context of the whole HPV-31 and HPV-16 genomes. Astonishingly, only minor phenotypic differences could be observed between E5 wild

type and mutant viruses, among them, in the case of mutant HPV-16 E5, a paucity of BrdU incorporating cells in the superficial layers of epithelial cells grown in raft cultures. Surprisingly, E5 mutants were found to continue to up-regulate the EGF receptor. Campo and colleagues (P-279) reported that the cloned *E5* gene of HPV-16, as that of BPV-1 and 4, reduces the expression of MHC 1, thereby contributing to the poor immune response to papillomavirus lesions.

4. The *E4* gene

Papillomaviruses do not lyse their host cells to achieve release into the extracellular space, but they are released by disintegration of superficial epithelial cells. From the work of John Doorbar's group we have known for many years that the E4 protein seems to be one of the viral tools to contribute to this process, namely to induce the collapse of the cytokeratin network of HPV infected cells. Here, we have learnt from the same group (Wang et al., P-304) that in the presence of the HPV-16 E1–E4 proteins cytokeratins form disorganized perinuclear bundles, similar to mutants of cytokeratin genes that lack the interaction sites with the cellular keratin solubility factors (14-3-3 proteins). An LLKLL motif at the N-terminus of the E1-E4 protein appears to be essential for binding keratin proteins.

E1-E4 proteins may have biological functions beyond their structural impact, as interactions with the replication factors Cdc6 and Mcm7 in a study by Knight and colleagues (P-303) and cyclin B-Cdc2 in a study by Davy and colleagues (O-174) pointed to a G2 arrest during the cell cycle. Peh and colleagues (O-146) reported on E4 mutants in the context of the full CRPV genome and their introduction into rabbits. The mutants generated superficially normal papillomas; they failed, however, to amplify the viral genome efficiently and to generate viral capsids suggesting functions of E4 in replication and late gene expression.

5. The capsid proteins: L1 and L2

The expression of L1 has many unconventional, not to say mysterious aspects. Among them is the diversity of promoters that give rise to L1 encoding mRNAs, making it difficult to substantiate the concept of an early-late switch, the efficient translation of L1, although it is encoded in downstream positions on polycistronic mRNAs, and the regulation of its transcript stability through a splicing donor site downstream 3′ to its coding sequence. In this meeting, Collier and colleagues (O-43) reported a 129 bp segment of L1 mRNA that interferes with efficient translation, and that can be mutated without altering the L1 protein sequence so as to allow high efficiency translation.

L1 is involved in binding the viral DNA, as Schäfer and colleagues (P-306) reported data that a C-terminal mutant can form capsids, together with L2 protein, that fail to incorporate DNA. The same group reported that HPV VLPs can incorporate naked as well as nucleosomally packaged DNA, and that both forms of viral particles are infectious. It was found, however, that the infectivity of the chromatin form was higher by a factor of five.

We still know fairly little about the infection process of papillomaviruses, from its attachment to the cell surface, the transport to the nucleus, and unpackaging reactions. For a number of years, alpha-6 integrin was considered to be an important candidate receptor to bind the papillomavirus particle. In this meeting, four groups (O-161, O-168, O-170, P-283) have amended this concept and shown that a variety of proteoglycans such as heparan sulfate appear to be the principal receptor for HPV-11, 16, 33, and BPV-1. Some of the data appear to contradict the involvement of alpha-integrin, and we have to wait for further research to understand whether one or multiple molecules function as cellular receptors for papillomaviruses.

Functions of the L2 protein, which is only a minor component of the papillomavirus capsid, have remained somewhat obscure for quite some time, among others, because capsids that contain or are free of L2 protein look indistinguishable in the electron microscope. Our ignorance about L2

changed a few years ago when it was found that L2 protein becomes localized to ND10 nuclear structures and triggers the co-localization of L1 and E2 protein. ND10 structures contain the proto-oncoprotein PML, and have important, although somewhat enigmatic functions in the life cycle of a variety of viruses. In this meeting, Florin and colleagues (O-179) confirmed that L2 protein of HPV-33 localizes to ND10 structures, but does so without altering the major component PML. However, it strongly downregulates the concentration of Sp100 and up regulates that of Daxx. As these proteins have transcriptional and pro- and anti-apoptotic functions, L2 can be increasingly seen as a player with regulatory functions in the viral life cycle beyond its role as a structural component of the capsid.

In the capsid, L2 may have a variety of functions possibly including binding of a secondary receptor, nuclear localization, or viral genome binding, as Roden and colleagues (O-178) reported on three alternative deletions of peptide sequences widely separated from one another, that render the mutant L2 protein wildtype in assays of virion assembly. These virions, however, are non-infective.

6. Viral diversity

The rate of detection of new HPV types appears to have accelerated instead of slowed down. Jin and colleagues (P-219) described eleven new presumed genital HPV types from cervical smears of HIV patients. Even more remarkable, Antonsson and colleagues published recently a paper on the observation of 30 DNA sequences derived from putative novel cutaneous HPV types during a screen of skin samples from Sweden. The same group extended these studies, and reported here (P-307, P-308) the detection of yet another 50 new HPV types from Bangladeshi, Japanese, and Swedish individuals. This whole group of viruses appears to be very widespread and is only occasionally found in lesions, such a warts caused by HPV-4, or the various cutaneous lesions of the epidermodysplasia verruciformis high-risk patients. These viruses are paradigms for evolution theories that argue that it is an essential part of viral evolution to become latently infecting commensals rather than following a strategy to cause disease. We should not forget that the same logic applies also to carcinogenic HPV types such as HPV-16, which is normally asymptomatic in the male, but is carcinogenic in the female genital tract, most often only in the special cellular context of the cervical transformation zone. Similarly, in this conference, Abramson and colleagues (P-285) pointed out that unknown cellular factors specific for certain tissue sites play a role in the etiology of laryngeal papillomas, as tracheal papillomas have a much lower incidence in spite of similar latent infection rates of tracheal tissue with HPV-6 and 11.

7. Cellular genes

The search for genes required for susceptibility and progression continues to be one of the biggest challenges of our field of research. Progress has been made in the research on the causes of EV, as Ramoz and colleagues (O-195) reported that the search for an EV susceptibility gene could be restricted to a relatively small 160 kb region on chromosome 17, which encodes two known and four putative genes.

The whole field of DNA microarray analysis is just beginning to be applied to HPV research. It has the potential to change the classification of HPV containing neoplasias based on molecular rather than on classical data, to lead to an understanding of the different molecular properties of different HPV types, and to help us to understand progression events. In the last year, publications by the groups of Laimins and Woodworth have opened this field, documenting, among others, the down-regulation by interferon-responsive genes by genital HPVs. In this conference, we have learnt of efforts by Oh and colleagues (O-163) to identify differential effects between HPV-11 and HPV-31, and by Kleine-Lowinski and colleagues (O-110) to study the differential effects of HPV-16 E6, E7, and E6/E7 in human foreskin keratinocytes, and of Yuan and colleagues (O-164), to combine the DNA microarray analysis with laser capture

microdissection to identify molecular subclasses of squamous cell carcinomas of the cervix. While still at early stages, these studies are without doubt opening a new chapter of HPV research.

The **Immunology** and the vaccine sessions during this meeting consisted of 33 oral presentations and 54 poster presentations. Apart from these sessions a lot of immunologically oriented presentations were apparent in the animal model and the therapy sessions bringing the immunology of HPV to a dominating presence in the 19th International Papillomavirus Conference in Florianopolis, Brazil. This presence indicates a remarkable increase in immunologically oriented presentations, since about 10 years ago only a handful of such presentations were presented at the international papillomavirus meeting. Despite this vast increase, no major hard-core immunological breakthroughs were presented at the meeting. This is not really surprising since current research relating to the immunology of HPV serves a purpose, namely: vaccine development, and is, therefore, primarily covering applied research aspects. A lot of work was presented on details that need to be analyzed to come to a final product, namely a preventive or a therapeutic vaccine. To put this formidable task into perspective one needs to realize that multiple different HPV types are linked to cancer, implying a need for a vaccine with a large combination of different HPV components for preventive purposes, and that no effective therapeutic vaccine for whatever disease is presently available. Reasons for the latter include an insufficient knowledge about the immune system, and the existence of tumor immune escape mechanisms. Thus the field of therapeutic vaccines for HPV associated cancers is running against the same wall as therapeutic vaccines for other cancers. However, if any therapeutic vaccine is going to be developed it will happen in this field where the cancer so blatantly expresses a foreign antigen, and in which it is clear that anti-HPV responses could and should make a major impact on tumor development and treatment. For now, the field has to go through an agonizing process of collecting all of the necessary details including which HPV proteins/epitopes are the right targets, what is the best vaccine adjuvant or vector, what is a safe approach, and how can one reduce the number of entities in a product. Safety is a major issue for therapeutic vaccines because it is likely that the viral oncogenes E6 and E7 need to be targeted, as they are the only two viral products consistently expressed in HPV-associated tumors.

Examples of reduction of the number of entities in a product were evident in this meeting by presentations from scientists from Medimmune and Merck that were analyzing hybrid HPV VLP that contained parts of multiple HPV types. Another problem that this field shares with other therapeutic cancer vaccine fields is in defining the surrogate immune markers that correlate with clinical outcome. Despite a slow progress overall in the immunology, vaccinology and immunotherapy of HPV associated lesions, there were gems in the presentations including: (1) The CRPV model data of Brandsma et al. on E6 containing vectors and DNA, reducing the growth rate and size of tumors, and even leading to some complete tumor regressions. (2) The data generated with alphavirus vectors that are likely safe delivery systems as they are based on an RNA approach, thus eliminating options for integration of *E6* and *E7* genes into the host genome. Daemen et al. using Semliki Forest virus vectors, Wyeth Lederle/Kast et al. using Venezuelan Equine Encephalitis virus vectors, and Wu et al. using Sindbis virus vectors all showed therapeutic capacities of their approaches in preclinical tumor models. (3) The first data of HPV proteins from transgenic plants thus far showing only moderate protection in animal models, yet also indicating the enormous potential of making a very cheap product, bringing vaccines within reach for underdeveloped countries. (4) The data from Wu et al. indicating that a MHC negative variant HPV positive tumor cell could be targeted with interferon gamma and NK cells. This provides hope for attacking tumors with down-regulated MHC, a phenomenon that is frequently observed in cervical cancer. (5) The data of Sanders et al. on cost effectiveness calculations is showing the cut offs for the economics of vaccines in relation to their effectiveness. (6) The intriguing data of Vambutas et al. on the possibly putative interaction of E7 with the transporter associated protein (TAP), possibly

resulting in antigen processing changes. (7) The data of Campo et al. on the surprising infiltration of HPV specific B cells into cervical cancer. (8) The data of Lewis et al. on the existence of a common mucosal immune response against HPV. (9) Stanley's overview on COPV research. Her suggestion years ago about the need for CD4 T cell research in the HPV field is being followed up by T helper cell epitope mapping, and by analysis of CD4 memory responses in patients and volunteers (Van der Burg et al.). (10) The data of Frazer et al. on the positive effects of codon optimization to better express HPV genes and on the production of HPV itself in yeast. This intriguing work could be the beginning of a second wave in preventive vaccines using inactivated whole virus instead of virus-like particles. (11) The encouraging data of StressGen reflecting results of their anal intraepithelial neoplasia (AIN) clinical trial with heatshock protein-HPV16E7 fusion proteins. AIN lesions are literally a pain in the behind and the high regression rates observed after vaccination are promising. Inexplicable among their data is the extremely high cross-reactivity of their HPV16E7 based vaccine towards other HPV types. As many as 34 out of 45 non-HPV16 patients showed regression or a lower anal SIL grade. Percentage wise, this was even higher than in the HPV16 positive AIN patients in which effects were seen in three out of seven patients. A question mark is also why one can observe lower SIL grades after treatment instead of regression. The clinical results warrant further testing and positive but aspecific adjuvant effects of this type of vaccination should be considered. (12) The data of Koutsky et al. reporting the first follow-up data of L1 VLP vaccinated volunteers. Two years after vaccination nine out of 129 controls showed evidence of HPV infection and zero out of 66 of the vaccinated group. These results were dubbed a historical moment at the meeting. Given the relatively small groups and early follow-up it might be better to report her data as 'pre' historical, but the promise of an effective preventive vaccine for HPV is clearly there. Some cautionary notes are also the Moscicki et al. data about pre-existing antibodies to HPV16 not protecting against HPV infection, the Wheeler et al. data on HPV antibody titers dropping considerably over time and the Nardelli et al. data on temporary drops in HPV antibody titers during ovulation, all of which would result in reduced protection of a preventive vaccine. The role of T cells in protection against HPV infection should also not be underestimated, but this aspect has not yet been studied.

The future for this field will deal with the following items: (1) preventive vaccines will have to prove that they really protect human beings and, (2) the keyword for therapeutic vaccines will be multi-modality treatments. Examples include inducing senescence or apoptosis via for instance anti sense E7 or aptamers to E6, all combined with the induction of immune responses. Cancer cells need to be hit in multiple ways to prevent escape from each of the individual treatments. The next International Papillomavirus Conference in October 2002 in Paris, France will likely lift a tip of the veil on these developments.

8. Recommended readings

Bosch, F.X., Lorincz, A., Munoz, A., Meijer, C.J.M., Shah, K.V., 2002. The causal relation between human papillomavirus and cervical cancer. J. Clin. Pathol. 55(4), 244–265.

Eiben, G.L., Velders, M.P., Kast, W.M., 2002. The cell mediated immune response against human papillomavirus induced cervical cancer: implications for immunotherapy. Adv. Cancer Res. 86, 113–148. Virus Res. 89 (2002).

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Gates Foundation, and the International Centre for Genetic Engineering and Biotechnology (ICGEB). The conference was supported by public grants from the National Institutes of Health. The conference was credited for CME purposes by the American Medical Association (AMA). The organizers acknowledge the support received from all sources and in particular those who diligently contributed manuscripts and gave outstanding presentations at the meeting. We would like to extend our gratitude to the staff of the LICR Sao Paulo branch, in particular Stella Leme Hering, the students and technicians of the Virology Group, and to JZ Congressos for an impeccable organization. Abstracts mentioned in the text are available at http://www.hpv2001.com.